

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Ian Garner, Michael A. Dalrymple, Donna E.  
Prunkard, Donald C. Foster  
Serial No. : 08/206,176  
Filed : March 3, 1994  
For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC  
ANIMALS

Examiner : Stanton, B.  
Art Unit : 1804  
Docket No.: 93-15  
Date : September 9, 1996

Assistant Commissioner for Patents  
Washington, D.C. 20231

*Considered  
12/18/96  
BAG*

Declaration of Will H. Eyestone Under 37 C.F.R. § 1.132

Sir:

I, Will H. Eyestone, hereby declare as follows:

1. I received a Ph.D. in animal science from the University of Wisconsin in 1989.

2. I am currently employed as Head of Bovine Technology at PPL Therapeutics, Inc., 1700 Kraft Drive, Blacksburg, Virginia.

3. I have read and understand the specification and claims of the above-identified application (the "Patent Application").

4. I have read the Office Action dated May 21, 1996 in the Patent Application, including the rejection of claims 2, 12, and 17 under 35 U.S.C. § 112, first paragraph, and provide this Declaration for the purpose of

assisting the Examiner in evaluating the teachings of the Patent Application.

5. As of the March 3, 1994 filing date of the Patent Application, the integration and expression of transgenes in cattle had been demonstrated and reported in the scientific literature. Also as of the filing date of the patent application, production of heterologous proteins in milk had been achieved in species closely related to cattle. In view of these data, there was at the time a reasonable expectation that a transgenic protein could be produced in the milk of cattle.

6. More specifically, production of transgenic cattle had been reported by Krimpenfort et al., Bio/Technology 9:844-847, 1991; Hill et al., Theriogenology 37:222, 1992; and Bowen et al., Biol. Reprod. 50:664-668, 1994. The Bowen et al. publication reported the expression of one transgene, c-ski. A calf transgenic for chicken c-ski showed a phenotype associated with expression of high levels of c-ski mRNA. In June 1994 Hyttinen et al. (Bio/Technology 12:606-608, 1994) also reported the birth of a transgenic calf. Copies of these publications are attached hereto as Exhibits 1-4.

7. It had also been shown that several diverse, heterologous proteins could be expressed in the milk of transgenic animals of several species, including ruminants (e.g., sheep and goats). Proteins that had been produced prior to March 3, 1994 include human  $\alpha_1$  antitrypsin, tissue plasminogen activator, and clotting factor IX.

8. The expression of heterologous proteins in milk had been achieved through the use of milk-specific promoters. The ovine beta-lactoglobulin promoter, for example, had been used to direct expression of heterologous proteins, including serum albumin and  $\alpha_1$  antitrypsin,

in at least two species, mice and sheep. These results, which were reported by Carver et al., Bio/Technology 11:1263-1270, 1993; Archibald et al., Proc. Natl. Acad. Sci. USA 87:5178-5182, 1990; and Shani et al., Transgenic Res. 1:195, 1992 (copies of which are attached hereto as Exhibits 5-7), indicate the utility of the ovine beta-lactoglobulin promoter in driving transgene expression in non-homologous species. It would have been expected that other milk-specific promoters would be similarly useful.

9. In view of experiments that had been done in transgenic animals and reported in the literature as of March 3, 1994, I readily conclude that the teachings of the Patent Application would have enabled one skilled in the art to generate transgenic cattle carrying expression units for the three chains of human fibrinogen. Those expression units would have included DNA segments required for the expression of the fibrinogen sequences in the mammary gland of a cow. These transgenic cattle would have been generated with the expectation that at least some female members of the resulting line would produce milk containing biocompetent fibrinogen encoded by the transgenic expression units.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may

jeopardize the validity of any patent issuing from this patent application.



Will H. Eyestone

Date: Sept. 23, 1996